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# ***Neuroimmune interactions in chronic pain – an interdisciplinary perspective***

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## **Abstract**

It is widely accepted that communication between the nervous and immune systems is involved in the development of chronic pain. At each level of the nervous system, immune cells have been reported to accompany and frequently mediate dysfunction of nociceptive circuitry; however the exact mechanisms are not fully understood. One way to speed up progress in this area is to increase interdisciplinary cross-talk. This review sets out to summarize what pain research has already learnt, or indeed might still learn, from examining peripheral and central nociceptive mechanisms using tools and perspectives from other fields like immunology, inflammation biology or the study of stress.

## **1. Introduction**

Over the past decade, it has become evident that the immune and nervous systems communicate closely in an effort to protect their host from injury. Chronic pain conditions are, more often than not, the result of such a conversation gone wrong (McMahon et al., 2015). Yet, historically, dialogue between neuroscience and immunology has been limited, and studies on neuroimmune interactions in chronic pain are usually designed by and for neuroscientists.

In this review, we make the case that our understanding of chronic pain could benefit from a more interdisciplinary perspective. Mechanistically, chronic pain ultimately arises because of dysfunction of the nociceptive circuitry at varying levels of the nervous system, resulting in a net increase in output and the percept of pain. At every one of these levels, immune cells have been reported to accompany this dysfunction and frequently even to mediate it. For instance, peripheral sensory neurons are hypersensitive and/or ectopically active in pain states – a process thought to be driven by pro-inflammatory mediator release (Marchand et al., 2005). Similarly, interfering with the function of microglia, the resident macrophages of the central nervous system (CNS), has been shown by many independent groups to dampen or completely reverse the development of a pain state – at least in animal models (Guan et al., 2016; Inoue and Tsuda, 2018).

Many review articles have previously summarized the current state of evidence for neuroimmune interactions in chronic pain states (Austin and Moalem-Taylor, 2010; Mapplebeck et al., 2017; Marchand et al., 2005; McMahon et al., 2015; Rosen et al., 2016). In the following text we will therefore only give a brief overview, limiting our discussions to key findings, and instead give particular attention to aspects where a cross-disciplinary

perspective has helped the field move forward, or where we believe it could do so in the future (see Figure 1 for a summary).

## **2. Peripheral neuroimmune interactions**

Pain researchers classically distinguish between painful inflammatory and painful neuropathic conditions (Costigan et al., 2009), with neuroimmune interactions historically being considered in the context of the former (Marchand et al., 2005). However, over the past two decades, it has become apparent that inflammation is also present in neuropathic conditions, and may play a role in mediating them. Both activation of resident immune cells (e.g. macrophages, dendritic cells) and recruitment of circulating immune cells (e.g. neutrophils, T cells) has been observed in the nerve and dorsal root ganglia (DRG) of various animal models of peripheral nerve injury (PNI). Such evidence stems from immunohistochemical experiments, for example following chronic constriction injury (CCI) in rats (Hu et al., 2007) and partial sciatic nerve ligation (PSNL) in mice (Kim and Moalem-Taylor, 2011), but also more recently from flow cytometry of mouse DRG subjected to PSNL surgery (Lopes et al., 2017). Similarly, in patients with osteoarthritis (OA) of the knee, magnetic resonance imaging (MRI) has confirmed inflammation of synovial membranes; a pathology reportedly associated with increased pain (Baker et al., 2010; Hill et al., 2007; Ishijima et al., 2011; Neogi et al., 2016). The primary cell types involved have been noted as macrophages and T cells, however, B cells, plasma cells, mast cells, natural killer cells and dendritic cells have all also been found in higher numbers in OA patients than healthy controls (de Lange-Brokaar et al., 2012). In addition to a bewildering number of different immune cells, there are the wide variety of mediators they release. Such mediators are known to have the capacity to directly sensitise nociceptors and/or recruit other immune

cell types, which can in turn release an array of pro- and anti-inflammatory substances, a number of which have been associated with pain, both in OA and after PNI (Austin and Moalem-Taylor, 2010; Ellis and Bennett, 2013; Miller et al., 2014).

Despite this complexity, the bulk of the data on peripheral immune responses in neuropathic pain is limited to a small number of immune cell types and mediators. Most studies examine macrophage-neuron interactions (Shepherd et al., 2018; Vega-Avelaira et al., 2009), while cytokines and chemokines are the most prominently examined mediators, with many reports on tumour necrosis factor (TNF) (Sacerdote et al., 2008; Sommer et al., 2001; Üçeyler et al., 2007), interleukin-1 $\beta$  (IL-1 $\beta$ ) (Gui et al., 2016; Üçeyler et al., 2007) and the inflammasome components which promote cleavage into its mature form (Lopes et al., 2015). Detailed studies of immune cell kinetics are still rare, with only a handful having investigated the presence of different immune cell populations over an extended time course following PNI (Hu and McLachlan, 2002; Kim and Moalem-Taylor, 2011; Moalem et al., 2004). Even so, none have persisted past 12 weeks (Hu and McLachlan, 2002), when inflammation is still present. Thus there is still little knowledge of when exactly inflammation is resolved in the different pain states. This is despite the fact that molecules thought to be responsible for this resolution, known as resolvins, have been shown to be dysregulated in arthritis (Arnardottir et al., 2016) and have anti-nociceptive action inflammatory pain, e.g. (Oehler et al., 2017). Finally, there is a lot of work on how immune cells talk to sensory neurons, but the details of how sensory neurons talk back have traditionally been less well explored. It has been known since the late 19th century (Bayliss, 1901) that sensory and sympathetic neurons can cause “neurogenic inflammation”, i.e. stimulate vasodilation by releasing neuropeptides such as substance P or calcitonin gene-related peptide (CGRP) which act on vascular endothelial and smooth muscle cells (Green et

al., 1998; Sousa-Valente and Brain, 2018). But it is only recently that a growing body of evidence is starting to reveal that in various painful conditions, neuropeptides and neurotransmitters can directly influence innate and adaptive immune cell functions themselves (McMahon et al., 2015). For instance, one study from last year found that in a mouse model of the painful bacterial infection necrotizing fasciitis, neuronal release of CGRP is responsible for inhibiting recruitment of neutrophils and consequently bacterial clearance (Pinho-Ribeiro et al., 2018).

Our current neuroscientific approaches are thus slow to paint a complete picture of neuroimmune dysfunction in chronic pain. Worse still, it is not clear how accurately we are even modelling the neuroimmune interactions taking place in human conditions. Immunologists have long appreciated that different animal models, e.g. of arthritis, usually only represent some immunological aspects among many more that are present in human disease (Vincent et al., 2012). Similarly, the inflammatory response brought about by the surgical nature of many widely used models of neuropathic pain, e.g. injury of peripheral nerves via ligation, may mean the actual neuroimmune reaction taking place in human conditions is misrepresented. While development of more suitable models is not an easy feat, more wide-spread adoption of immunological techniques and nomenclature would also be very helpful and is easier to implement.

Technically, standard use of flow cytometry (FC) alongside traditional immunostaining experiments would be extremely beneficial for the field. FC can simultaneously detect numerous markers, allowing for high throughput quantification. Furthermore, it enables immunophenotyping of cells based on both surface and intracellular markers and, in combination with cell sorting, the generation of cell-type specific transcriptional profiles. To

date, publications in the pain field too frequently neglect to perform FC or do not perform it correctly. Many studies fail to compensate for potential overlap between fluorochrome emissions, rendering the experiment meaningless. Even more often, gating strategies are not published and appropriate staining techniques are not employed, e.g. with the omission of Fc block or live/dead staining likely resulting in high background noise.

Even more simply, pain research could benefit from considering peripheral neuroimmune interactions in more complex terms – keeping up a public consciousness of the myriad of different immune cell types, phenotypes and kinetic relationships that are likely to be involved. An easy start could for instance involve abandoning the traditional binary M1/M2 classification of macrophages in favour of a new consensus nomenclature based on activation standards e.g. M(IFN- $\gamma$ ), M(IL-4), M(LPS), M(IL-10) and so on (Murray et al., 2014), which would make it easier to standardise findings across studies.

Interdisciplinary perspective in pre-clinical studies will likely benefit clinical work, and help improve our understanding of puzzling phenomena, such as why resolution of inflammation often fails to reset the nociceptive signalling system. Rheumatoid arthritis (RA) is one such example. This chronic autoimmune disease causes swelling of the joints, resulting in pain, stiffness, loss of function and, over time, damage to the joint itself. Pain in RA is deeply debilitating for patients (Klooster et al., 2007), but was long considered a simple by-product of inflammatory processes that would remit once the auto-immune response could be prevented.

However, this turns out not to be the case. Many patients report pain even when their disease activity is at a minimum (Altawil et al., 2016; Lee et al., 2014) or in remission (Lee et al., 2011). This is true even for patients on newer disease-modifying anti-rheumatic drugs (DMARDs), such as anti-TNF (Andersson et al., 2017; Klooster et al., 2007). This

uncoupling of pain and inflammation has also been highlighted in animal models of RA, where numerous studies have shown that mechanical hypersensitivity occurs both before onset and after resolution of inflammation (Bas et al., 2012; Christianson et al., 2011; Clark et al., 2012; Nieto et al., 2016). Finally, while the majority of OA patients benefit from knee replacement surgery (Beswick et al., 2012), it has been reported that a disconnect between resolution of inflammation and pain can be observed in some individuals (Neogi et al., 2016).

There are several potential mechanisms that could explain these observations that are by no means mutually exclusive. A simple one is that inflammation is required to initiate sensitization of peripheral nociceptors, but not to sustain it. Another possibility that likely explains continued pain in a number of patients is that the CNS has now become independently sensitized. Once more however, immune cells have been heavily implicated in this process – as we will discuss in the following.

### **3. Spinal cord neuroimmune interactions**

At the level of the spinal cord, various pain conditions are well known to cause increased excitability and synaptic efficacy of CNS neurons – a phenomenon known as central sensitization (Woolf, 2011). Microglia are likely to be important for this process, with evidence for their involvement in animal models of several neuropathic pain conditions (Calvo and Bennett, 2012). For example, spinal microglial activation, assessed using immunohistochemical techniques, has been observed in rats following both PSNL (Clark et al., 2007) and CCI (Hu et al., 2007), but also extensively in various animal models of RA. These include collagen antibody–induced arthritis (CAIA) (Bas et al., 2012), collagen-induced arthritis (CIA) (Clark et al., 2012; Nieto et al., 2016) and K/BxN serum transfer arthritis



(Christianson et al., 2011), where significantly higher numbers of microglia, as measured by expression of the myeloid cell marker ionized calcium-binding adapter molecule 1 (IBA-1), have been observed in the dorsal horns of arthritic animals compared to controls. Indeed, pain models are a great tool with which to study microglial activation; inducing such intense spinal proliferation of these cells as to be easily detectable by the naked eye.

And yet, the exact mechanisms by which microglia and other immune cells contribute to chronic pain states at spinal cord level are still being debated. Interdisciplinary knowledge has helped clarify some of these questions and has the potential to resolve many more. For example, it has been proposed that peripheral nerve injury (PNI) causes opening of the blood-spinal cord barrier and subsequent infiltration of blood circulating myeloid cells into the spinal cord parenchyma (Beggs et al., 2010; Echeverry et al., 2011), where they contribute to spinal microgliosis (Zhang et al., 2007). However, recent work using flow cytometry (Denk et al., 2016) and/or transgenic approaches (Gu et al., 2016; Tashima et al., 2016) has found no significant infiltration of monocytes into the spinal cord following PNI. For instance, this has been demonstrated using bone marrow chimeric mice which were generated without irradiation, thus avoiding disruption to the blood brain barrier (BBB) (Tashima et al., 2016), in contrast to the techniques utilised by several other groups reporting infiltration (Echeverry et al., 2011; Isami et al., 2013; Zhang et al., 2007). Likewise, use of myeloid-lineage reporter mouse lines has indicated that, following spinal nerve transection (SNT), spinal microgliosis results primarily from local proliferation of resident microglia, as opposed to infiltrating monocytes (Gu et al., 2016). This observation also extends to mouse models of RA, where the use of flow cytometry has revealed macrophage infiltration in the lumbar dorsal horn to be negligible up until at least day 19 of the inflammatory phase (Fernandez-Zafra et al., 2018). Thus, the simple import of

immunological techniques produced strong evidence for microgliosis being solely reliant on the proliferation of resident microglia and shifted the prevailing view within the pain field on this topic within a very short space of time (Mapplebeck et al., 2017).

Beyond this, there is still uncertainty about how exactly injured sensory neurons initiate recruitment of these resident microglia. Involvement of various neuron-derived factors has been reported (Calvo and Bennett, 2012), and more recently, cytokine colony-stimulating factor 1 (CSF1) and the microglial membrane adaptor protein DAP12 have emerged as promising candidates necessary for proliferation and activation of microglia, respectively (Guan et al., 2016). Similarly, it is still not fully established how microglia, in turn, sensitize neurons. Several mechanisms have been put forward in the literature, such as release of cytokines (Clark et al., 2013), reactive oxygen species (Kim et al., 2010), microglial-derived inflammasomes (Grace et al., 2016) or brain-derived neurotrophic factor (BDNF) (Coull et al., 2005). However, many questions remain. For instance, BDNF is reportedly released upon ATP activation of microglia via the P2X4 receptor and alters the excitability of neurons through binding of TrkB receptors (Beggs et al., 2012). However, numerous cell-type specific RNA sequencing (RNA-seq) experiments have revealed that microglia do not express BDNF message at significant levels, neither in the cortex (Gosselin et al., 2014; Lavin et al., 2014; Zhang et al., 2014), nor in healthy spinal cord (Fernandez-Zafra et al., 2018; Matcovitch-Natan et al., 2016), nor in spinal cord after nerve injury (Denk et al., 2016; Tay et al., 2018). These data are yet another example of how integration of findings beyond the narrow discipline of pain research has helped open up new questions and avenues of investigation.

Another area where interdisciplinary information is starting to aid our understanding is in the characterisation of microglial activation states. It has become evident that microglia are

highly plastic and can take on a multitude of stimulus-dependent phenotypes that transcend the simple duality of 'resting' versus 'activated' (Town et al., 2005). Tools are starting to be developed to help make sense of this heterogeneity, both at a morphological (Davis et al., 2017) and transcriptional level. Regarding the latter, RNA-seq data suggest there could be a common microglial activation signature, both in homeostasis (Butovsky et al., 2014) and in disease states (Butovsky and Weiner, 2018). For instance, one can pinpoint a transcriptional signature of neurodegeneration that is common to amyotrophic lateral sclerosis, Alzheimer's disease and multiple sclerosis (Butovsky and Weiner, 2018). While it remains to be formally determined whether similar overlaps can be observed in injury states, relevant data are already available from animal models of PNI (Denk et al., 2016), spinal cord injury (Noristani et al., 2017) and facial nerve axotomy (Tay et al., 2018).

Considering microglia are the primary resident immune cells of the CNS, it makes sense that they have been at the forefront of investigations into spinal cord neuroimmune interactions, but are they always involved? Over the past few years, a debate has arisen in the field regarding a differential role for microglia in contributing to pain states in male versus female mice. The data remain somewhat contradictory, possibly due to differences in species and models used (Sorge and Strath, 2018), but one frequently replicated finding is the ability of the antibiotic minocycline to reverse microglial activation and hypersensitivity in male, but not female mice (Chen et al., 2018; Fernandez-Zafra et al., 2018; Sorge et al., 2015). It has been suggested that T cells might be responsible for pain behaviour in females (Sorge et al., 2015), but the mechanisms for this remain unclear given that very few T cells are detectable in spinal cord after injury, neither via immunostaining (Gattlen et al., 2016; Kim and Moalem-Taylor, 2011) nor flow cytometry (Denk et al., 2016; Lopes et al., 2017). Microglial proliferation does not appear to differ after nerve injury in male versus female rodents

(Lopes et al., 2017; Nieto et al., 2016; Sorge et al., 2015), though flow cytometry points to possible minor variations in microglial numbers in naïve lumbar spinal cord that can be uncovered with high n numbers (n = 10-11) (Fernandez-Zafra et al., 2018). Transcriptional sex differences also appear to be very subtle at baseline (Fernandez-Zafra et al., 2018) – two recent studies on cortical microglia presenting results to the contrary (Guneykaya et al., 2018; Villa et al., 2018) relied on sorted cell populations which were clearly contaminated with neutrophils and neurons/astrocytes, respectively. Once again, a peek across the boundary between neuroscience and immunology could help untangle some of these results. To begin with, it would make it clear that sex differences in immune function are not in the least unexpected, but indeed have been recorded over decades to influence both innate and adaptive immunity (Klein and Flanagan, 2016). They are unlikely to be as drastic as perhaps originally proposed in our field – pitting one entire cell type against another – but they are probably ubiquitous: indeed, in the periphery, even simple differences in T cell numbers can be readily observed after PNI (Lopes et al., 2017).

#### **4. Brain neuroimmune interactions**

A final, potentially important location for neuroimmune interactions is the brain itself, where the pain percept is ultimately generated (Denk et al., 2014). It is well established that brain microglia are involved in homeostasis and host defence, however their role in disease states, like acute brain injury or chronic neurodegenerative disorders is still being debated. They have variably been reported to ameliorate as well as exacerbate the progression of a number of disorders including Alzheimer's disease, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis (Hickman et al., 2018; Perry et al., 2010). Meanwhile, evidence from animal models of acute brain injury has indicated that microglia, although

essential for synaptic regeneration due to their scavenging properties, also increase lesion size (Perry et al., 2010). Within the pain field, work focusing on this area has been rare, meaning mechanisms are even less well understood. Pre-clinical studies are generally restricted to immunohistochemical explorations of microglial numbers, based on IBA-1 expression. Although sparse, findings from such studies have generally mirrored those of spinal microglia, with two relatively recent papers pointing to changes in both microglial activation state (Taylor et al., 2017) and density (Chu Sin Chung et al., 2017) in pain-relevant brain areas following PNI, including the thalamus, prefrontal cortex and amygdala. Likewise, it has been demonstrated that there is a significant increase in the density of IBA-1 positive cells in several pain-related brain regions, including the anterior cingulate cortex (ACC) and periaqueductal gray (PAG), in a model of chemotherapy-induced peripheral neuropathy (CIPN), induced by administration of oxaliplatin (Di Cesare Mannelli et al., 2013).

Clinically, the few experiments that have been conducted suggest that cortical neuroimmune interactions could contribute to patient phenotype – at least in certain pain states. For instance, syndromes such as fibromyalgia (FM) (Gur and Oktayoglu, 2008) and chronic fatigue syndrome (CFS) (Montoya et al., 2017) are widely believed to have some immune involvement, including in the CNS. For instance, elevated levels of the known glial activation marker, translocator protein (TSPO) have been reported in both FM and CFS (Albrecht et al., 2019; Nakatomi et al., 2014), as well as in lower back pain (Loggia et al., 2015) patients. However, it should be noted that there are known limitations in both the sensitivity and specificity of TSPO binding ligands (Turkheimer et al., 2015; Venneti et al., 2013). For example, there is evidence that TSPO is also upregulated in activated astrocytes (Lavis et al., 2012), and that TSPO polymorphisms can alter ligand uptake (Kreisl et al., 2013) – questioning its reliance as a readout of microglial activation alone. Furthermore, its

usefulness is hindered by the fact TSPO targeting radioligands do not currently allow for distinction between anti- and pro-inflammatory phenotypes (Airas et al., 2018).

One important question to ask in this context is how we can accurately dissociate the role of CNS immune cells in generating pain versus other, related symptoms. Considering chronic pain is accompanied by various comorbidities, including depression and anxiety (Nicholson and Verma, 2004), which reportedly develop as a result of stress-induced microglial activation in the brain (Wang et al., 2018), this appears challenging. On the other hand, this tight link to other conditions could also present an opportunity: we may be able to gain a better understanding of potential neuroimmune mechanisms by looking at research in other areas of neuroscience. For instance, the co-occurrence of chronic stress and hyperalgesia is well noted (Jennings et al., 2014). And of course, we know a lot about how the CNS interacts with the immune system under stressful conditions. It is clear that early-life stress influences the peripheral immune system through the hypothalamic–pituitary–adrenal (HPA) axis and central neurodevelopment through microglial dysfunction (Johnson and Kaffman, 2018). For instance, stressors ‘prime’ microglia to respond differently to subsequent insults (Frank et al., 2007). These processes also seem to be crucial in the interaction between stress and aging (Fonken et al., 2018) – maybe of particular relevance to chronic pain conditions, given that they are much more likely to occur in the elderly.

There is also a lot of work on cellular stress, e.g. that caused by brain injury after stroke (Anthony et al., 2012), which once more could be interesting to consider in the context of pain. For instance, stroke can lead to both systemic immune activation and suppression. Large infarct volumes cause an acute phase response, where the CNS communicates damage to the liver which releases acute phase proteins. One such protein is C-reactive

protein (CRP), which is found at increased levels in the blood in the presence of inflammation. Conversely, stroke is also often followed by so-called stroke-induced immunosuppression – a process that is possibly mediated by catecholamines released from the autonomic nervous system and leads to increased incidence of conditions such as pneumonia post-stroke. In chronic pain patients, high CRP levels are usually only found as a result of systemic inflammation, e.g. in RA. In fact, they have been suggested as a way to distinguish RA patients from those suffering with FM (Hauser et al., 2017). Nevertheless, it is conceivable that the brain of a long-term pain patient does engage similar systems of communication with the body, though to a much lesser degree: subtly influencing peripheral or central immune function over long periods of time.

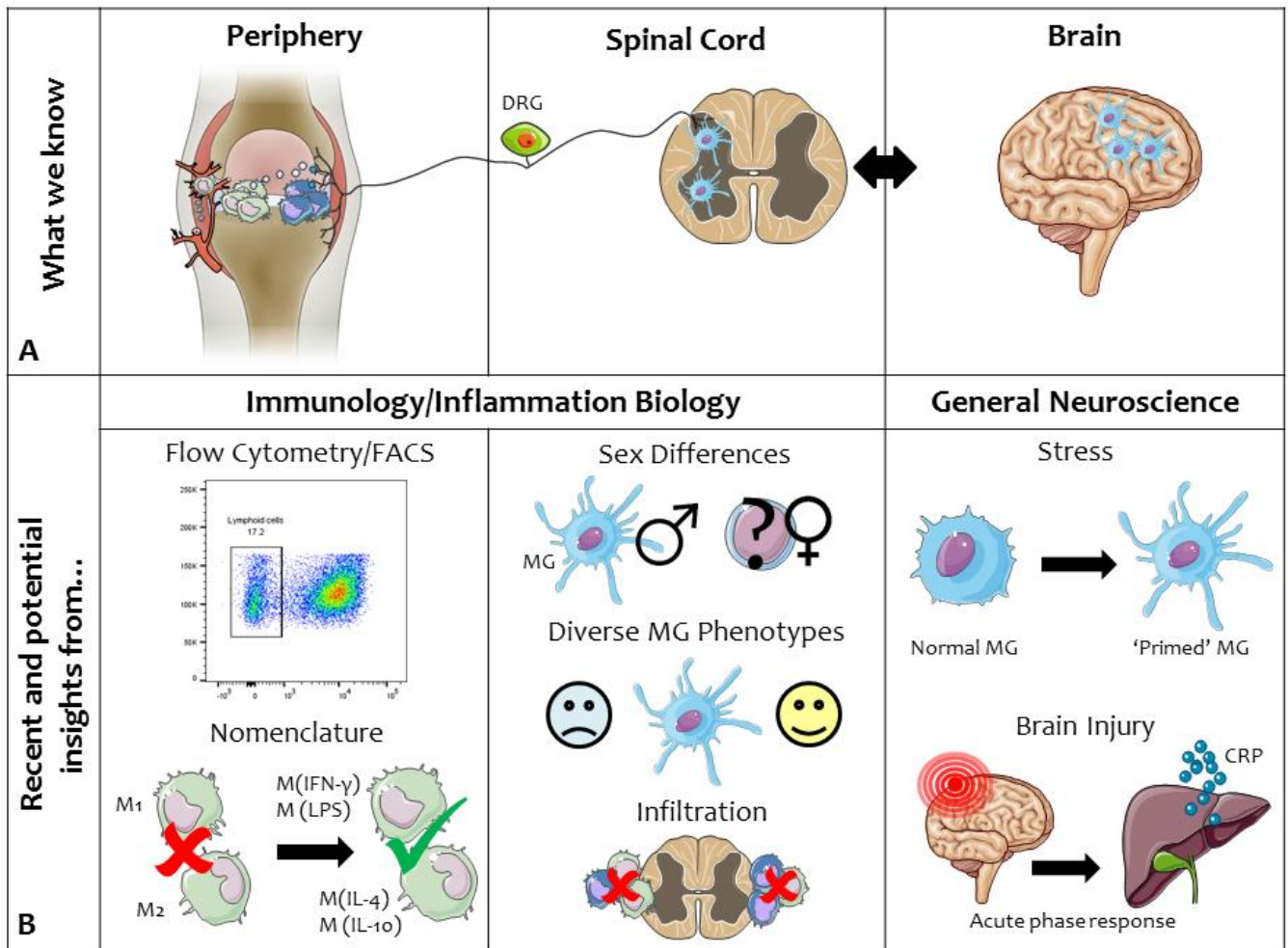
## **5. Conclusion**

In conclusion, we hope to have demonstrated that the use of interdisciplinary tools and knowledge could greatly aid our understanding of neuroimmune interactions in chronic pain.

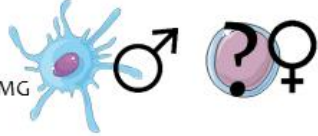
Of particular note, increasing dialogue with scientists from immunology and inflammation biology will likely help dissect complex peripheral immune responses. It could increase appropriate use of immunological techniques, such as flow cytometry and fluorescence-activated cell sorting (FACS), along with adoption of revised nomenclature to improve standardisation of findings. Centrally, looking to the immunology field could further our knowledge about the role of microglia in chronic pain; and taking a step back to consider what is already known about neuroimmune interactions in other areas of neuroscience, such as stress and brain injury, also poses a potentially beneficial avenue.

Interdisciplinary approaches are already starting to gain momentum, e.g. use of flow cytometry has helped us to characterise various aspects of the immune response to painful nerve injury. However, there are still many unanswered questions that could be solved by moving further outside of our comfort zone. While the sheer number of scientific publications issued month by month can frequently be overwhelming, they also present a unique opportunity – to be inspired by and start an internal dialogue with research outside the pain field and the neurosciences in general.







Sex Differences



Diverse MG Phenotypes



Infiltration



**Figure 1:** A| An overview of well-established neuroimmune interactions involved in the generation of chronic pain: peripheral immune response to injury, involving release of damaging mediators; proliferation/activation of microglia in the spinal cord and brain. *DRG*, dorsal root ganglion.

B| Examples of how insights from immunology, inflammation biology and general neuroscience have increased or have the potential to increase our knowledge of how neuroimmune mechanisms contribute to chronic pain states. *FACS*, fluorescence-activated cell sorting; *M*, macrophage; *MG*, microglia; *CRP*, C-reactive protein.

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